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M-a-85 (Revision #10)
May 30, 2003

TO: All Regional Food and Drug Directors
Attn: Regional Milk Specialists

FROM: Milk Safety Branch (HFS-626)

SUBJECT: Beta lactam Test Methods for Use Under Appendix N and Section 6 of the
Pasteurized Milk Ordinance (PMO)

This coded memorandum replaces and rescinds the previous edition of this coded memorandum (M-a-85-Revision #9).

This revision is based on actions taken by the National Conference on Interstate Milk Shipments' (NCIMS) Executive Board following a Laboratory Committee Report, addressing the acceptance of two new Beta lactam test methods (Charm SL6™ and IDEXX New Snap BL), given at an Executive Board meeting held at the 2003 NCIMS Conference, in Seattle, WA, and the phasing out of two (2) tests.

The individual Test tables presented in this revision provide data points that were derived from testing at least thirty (30) samples at each concentration for each drug detected.

This revision modifies the tables to reflect the addition of the Charm SL6™ and IDEXX New Snap BL Tests. It also reflects the removal of the Charm I/Cowside II Tablet Beta Lactam Test and Charm I/Cowside II Test for Cloxacillin in Milk (Competitive Assay) Screening Kits as the manufacturer has stated that these tests are obsolete and no longer in use for Appendix N and Section 6 testing. For information regarding these tests please refer to M-a-85 (Revision #9) to see how these tests performed.

The attached information is summarized from the evaluation of data submitted by test sponsors. Information related to the protocol used in this evaluation is available from Dr. Linda D. Youngman, FDA's Center for Veterinary Medicine (CVM), (301) 827-8010. Additional information regarding performance of these tests may be available from the test kit manufacturers.

Label claims for these tests were evaluated for use on raw commingled bovine milk samples. The evaluation protocol did not measure the performance of these tests in the assay of drug residues in other milk matrices, i.e., pasteurized milk or milk taken from individual cows, although claims for such use are made by some of the manufacturers of these tests.

NOTE: FDA recognizes that six (6) Beta lactams are widely used in treating disease in lactating dairy cattle and are the most likely to cause a residue in milk if misused. These are penicillin, ceftiofur, cloxacillin, cephalixin, amoxicillin, and ampicillin. While it is preferred that monitoring for Beta lactams include all of these drugs, at this time, the Agency is recommending that methods be utilized that have been shown to detect at least four (4) of the six (6) Beta lactams identified above.

Testing for drug residue(s) in compliance with the provisions of Sections 6 and 7 of the PMO may be accomplished by the use of any accepted Appendix N test for raw milk or an accepted Section 6 test for raw and pasteurized milk.

The NCIMS Executive Board has agreed that future updates to M-a-85 that add, delete or revise these tests will not require a public comment periods or follow the protocol established in the Procedures document for the issuance of M-a's.

Copies of this coded memorandum are enclosed for distribution to Regional Milk Specialists, State Milk Regulatory Agencies, State Laboratory Evaluation Officers and State Milk Sanitation Rating Officers in your region. This memorandum will also be available on the FDA Web site at <http://www.cfsan.fda.gov> at a later date and should be widely distributed to representatives of the dairy industry, State Veterinarians, State Veterinary and Pharmacy Boards, Veterinarian Professional Organizations and other interested parties.

/ss/

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/ss/

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ATTACHMENT TO M-a-85 (REVISION #10)

MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS ¹
BETA lactams

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE OR SAFE LEVEL	10 ppb	10 ppb	50 ppb ²	20 ppb	10 ppb	5 ppb
SCREENING TEST						
CHARM <i>B. stearothermophilus</i> TABLET DISK ASSAY ^{12, 14}	7.5	6.7	75 ⁷	11.7	48 ⁷	3.8
CHARM II TABLET BETA lactam TEST (COMPETITIVE ASSAY) ¹²	7.5	5.7	7.1	4.2	70 ⁷	3.0
CHARM II TABLET BETA lactam TEST (SEQUENTIAL ASSAY)	8.1	6.6	11.3	4.1	50 ⁷	3.4
CHARM SL BETA lactam TEST ¹¹	5.6	8.5	46.2	13.7	50 ⁷	3.6
CHARM SL6 TM BETA lactam TEST	7.1	9.6	37.5	18.7	8.3	4.2
CHARM II TABLET BETA lactam TEST (QUANTITATIVE ASSAY) ³	8.1	6.6	11.3	4.1	8.5	3.4
CHARM II TABLET TRANSIT BETA lactam TEST ⁴	7.5	5.7	7.1	4.2	50 ⁷	4.5
CHARM II TEST FOR CLOXACILLIN IN MILK (COMPETITIVE ASSAY) ^{5,12}	ND ⁶	ND ⁶	ND ⁶	ND ⁶	8.5	ND ⁶
DELVOTEST P 5 PACK ^{12, 13, 14}	4.6	4.0	NA ⁸	8.2	NA ⁸	2.1
DELVOTEST P/DELVOTEST P MINI ¹⁴	7.7	5.1	>50	7.0	30 ⁷	3.1
DELVOTEST SP/DELVOTEST SP MINI	6.0	7.9	>50	7.7	33 ⁷	2.7
DELVO-X-PRESS BETA lactam RESIDUE TEST KIT	9.2	7.0	11.5	4.1	50 ⁷	4.7
PENZYME MILK TEST ¹⁴	6.0	7.0	80 ⁷	11.6	80 ⁷	5.0
PENZYME III TEST PROCEDURE ¹⁴	5.3	5.6	NA ⁸	14.3	NA ⁸	4.3
SNAP BETA lactam TEST KIT (READER) ^{9, 12}	8	8.2	10.5	3.5	50 ⁷	3.5
SNAP BETA lactam TEST KIT (VISUAL) ^{9, 12, 14}	9.4	8.2	9.6	3.0	50 ⁷	3.4
New SNAP BETA lactam TEST KIT ⁹	7.3	5.8	5.4	11.7	50 ⁷	3.0
PARALLUX BETA lactam ASSAY	3.6 ¹⁰	2.9 ¹⁰	33.7	16.3	7.4	3.2 ¹⁰

Continued on next page

<p style="text-align: center;">CONTINUED: MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS¹ BETA lactams</p>
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FOOTNOTES:

1. Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following tables and should be considered when selecting drug residue monitoring tests. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.
2. Parent Drug
3. Test sensitivity when presumptive positive milk samples are verified in accordance with label directions using the Charm II Tablet Sequential Assay and the Charm II Test for Cloxacillin in Milk.
4. The Charm II Tablet Transit Test requires the use of a composite sample. Sampling techniques used to obtain these samples should be evaluated and accepted on a State-by-State or case-by-case basis.
5. For Appendix N bulk milk tanker screening, this test must be used in combination with other approved screening methods in order to detect at least four (4) of the six (6) targeted Beta lactam drugs.
6. ND indicates "Not Detected"
7. 90/95% concentrations were not determined for sensitivities significantly above the tolerance/safe level.
8. NA indicates "Data Not Available"
9. The SNAP Beta lactam Test is being replaced by the New SNAP Beta lactam Test. The visual reading option is not available with the New SNAP.
10. For Parallax tests, due to significant cross reactivity of cloxacillin in the Cillins channel, a positive Cillins test may be due to cloxacillin.
11. The Charm SL Beta lactam Test is acceptable for testing raw, commingled goat milk (M-I-03-3).
12. This test is acceptable for use to detect Beta lactam residues when used with pasteurized whole milk and pasteurized skim milk.
13. This test is acceptable to detect ampicillin, amoxicillin, cephalixin and penicillin residues in fat-free chocolate, whole chocolate, half & half, heavy cream and pasteurized goat milk.
14. Refer to M-I-01-4 for certification requirements to use this visual test.

CHARM *B. stearotheophilus* TABLET DISK ASSAY
DRUG CONCENTRATION RESPONSE ^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				0
2	0	0	0	0
3				0
4	10	3	0	55
5				100
6	30	67		
8	90	100	0	
10	100	100		
14			100	
20			100	

¹ Percent positive

² Based on 30 samples at each concentration

CHARM II TABLET BETA lactam TEST (COMPETITIVE ASSAY)
DRUG CONCENTRATION RESPONSE ^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	5
DRUG CONCENTRATION (ppb)					
1					10
2	3	3		30	67
3					97
4	10	43		100	100
5			60		100
6	83	97			
8	100	100		100	
10	100	100	100		
14				100	
20			100	100	
30			100		
50			100		

¹ Percent positive

² Based on 30 samples at each concentration

³ Parent drug

CHARM II TABLET BETA lactam TEST (SEQUENTIAL ASSAY)
DRUG CONCENTRATION RESPONSE ^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	5
DRUG CONCENTRATION (ppb)					
1					0
2	0	0		3	10
3					80
4	20	10		100	100
5			0		100
6	23	83			
8	93	97		100	
10	100	100	83		
14				100	
20			100	100	
30			100		
50			100		

¹ Percent positive

² Based on 30 samples at each concentration

³ Parent drug

CHARM II TABLET BETA lactam TEST (QUANTITATIVE ASSAY)
DRUG CONCENTRATION RESPONSE ^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXICILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	10	5
DRUG CONCENTRATION (ppb)						
1						0
2	0	0		3	0	10
3						80
4	20	10		100	3	100
5			0			100
6	23	83			17	
8	93	97		100	87	
10	100	100	83		100	
14				100		
20			100	100		
30			100			
50			100			

¹ Percent positive

² Based on 30 samples at each concentration

³ Parent drug

**CHARM II TABLET TRANSIT BETA lactam TEST
DRUG CONCENTRATION RESPONSE ^{1,2}**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	5
DRUG CONCENTRATION (ppb)					
1					10
2	3	3		30	67
3					97
4	10	43		100	100
5			60		100
6	83	97			
8	100	100		100	
10	100	100	100		
14				100	
20			100	100	
30			100		
50			100		

¹ Percent positive

² Based on 30 samples at each concentration

³ Parent drug

**CHARM II TEST FOR CLOXACILLIN (COMPETITIVE ASSAY)
DRUG CONCENTRATION RESPONSE ^{1,2}**

DRUG	CLOXACILLIN
TOLERANCE/SAFE LEVEL (ppb)	10
DRUG CONCENTRATION (ppb)	
2	0
4	3
6	17
8	87
10	100

¹ Percent positive

² Based on 30 samples at each concentration

CHARM SL BETA lactam TEST
DRUG CONCENTRATION RESPONSE^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	5
DRUG CONCENTRATION (ppb)					
1					0
2	3	3			13
3					73
4	70	13		0	100
5					100
6	100	83			
8	100	100		50	
10	100	97	0		
12				97	
16				100	
20			0	100	
30			13		
40			70		
50			100		

¹ Percent positive

² Based on 30 samples at each concentration

³ Parent drug

CHARM SL6™ BETA lactam TEST
DRUG CONCENTRATION RESPONSE^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	10	5
DRUG CONCENTRATION (ppb)						
1						7
2	0	0			0	23
3						93
4	7	3		0	40	100
5						100
6	57	17			83	
8	97	77		40	97	
10	100	100	0		100	
12				53		
16				100		
20			27	100		
30			73			
40			93			
50			100			

¹ Percent positive

² Based on 30 samples at each concentration

³ Parent drug

**DELVOTEST P 5 PACK
DRUG CONCENTRATION RESPONSE ^{1,2}**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				3
2	10	7	3	60
3				100
4	100	97	100	100
5				100
6	100	100		
8	100	100	100	
10	100	100		
14			100	
20			100	

¹ Percent positive

² Based on 30 samples at each concentration

**DELVOTEST P/DELVOTEST P MINI
DRUG CONCENTRATION RESPONSE ^{1,2}**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				0
2	33	3	0	0
3				100
4	47	70	7	100
5				100
6	93	100		
8	97	100	100	
10	100	97		
14			100	
20			100	

¹ Percent positive

² Based on 30 samples at each concentration

**DELVOTEST SP/DELVOTEST SP MINI
DRUG CONCENTRATION RESPONSE^{1,2}**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				7
2	0	0	3	47
3				100
4	80	10	17	100
5		33		100
6	100			
8	100	100	100	
10	97	100		
14			100	
20			100	

¹ Percent positive

² Based on 30 samples at each concentration

DELVOTEST-X-PRESS BETA lactam RESIDUE TEST KIT
DRUG CONCENTRATION RESPONSE^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	5
DRUG CONCENTRATION (ppb)					
1					17
2	17	20		57	57
3					73
4	47	70		97	87
5			93		100
6	80	90			
8	93	97		100	
10	97	100	97		
14				100	
20			97	100	
50			100		

¹ Percent positive

² Based on 30 samples at each concentration

³ Parent drug

PENZYME MILK TEST
DRUG CONCENTRATION RESPONSE^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				0
2	10	3	7	0
3				33
4	97	80	83	97
5				100
6	100	100		
8	100	100	100	
10	100	100		
14			100	
20			100	

¹ Percent positive

² Based on 30 samples at each concentration

**PENZYME III TEST PROCEDURE
DRUG CONCENTRATION RESPONSE ^{1,2}**

DRUG	AMOXICILLIN	AMPICILLIN	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	20	5
DRUG CONCENTRATION (ppb)				
1				0
2	7	43	10	33
3				77
4	77	97	87	97
5				100
6	100	100		
8	100	100	100	
10	100	100		
12			100	
20			100	

¹ Percent positive

² Based on 30 samples at each concentration

**SNAP BETA lactam TEST KIT (READER)
DRUG CONCENTRATION RESPONSE ^{1,2,3}**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ⁴	20	5
DRUG CONCENTRATION (ppb)					
1				10	7
2				93	80
3	0	30		100	100
4	3	93	90	100	97
5				100	100
6	90	100	100		
8	100	100	100		
10	100	100	100		
12			100		

¹ Percent positive

² Based on 30 samples at each concentration

³ The SNAP BETA lactam TEST is being replaced by the New SNAP BETA lactam TEST.

⁴ Parent drug

SNAP BETA lactam TEST KIT (VISUAL)
DRUG CONCENTRATION RESPONSE ^{1,2,3}

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ⁴	20	5
DRUG CONCENTRATION (ppb)					
1				20	7
2		27		100	80
3	0			100	100
4	0	90	77	100	97
5				100	100
6	40	100	100		
8	80	100	100		
10	100	100	100		
12			100		

¹ Percent positive

² Based on 30 samples at each concentration

³The SNAP BETA lactam TEST is being replaced by the New SNAP BETA lactam TEST. The visual reading option is not available with the New SNAP.

⁴Parent drug

NEW SNAP BETA lactam TEST KIT
DRUG CONCENTRATION RESPONSE ^{1,2}

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	5
DRUG CONCENTRATION (ppb)					
1					7
2	0	0		0	37
3					93
4	20	37		0	100
5					100
6	70	100			
8	100	100		0	
10	100	100	87		
12				100	
20			100	100	
30			100		
40			100		
50			100		

¹ Percent positive

² Based on 30 samples at each concentration

³ Parent drug

**PARALLUX BETA lactam ASSAY
DRUG CONCENTRATION RESPONSE ^{1,2}**

DRUG	AMOXICILLIN	AMPICILLIN	CEFTIOFUR	CEPHAPIRIN	CLOXACILLIN	PENICILLIN
TOLERANCE/SAFE LEVEL (ppb)	10	10	50 ³	20	10	5
DRUG CONCENTRATION (ppb)						
1						3
2	30	70		0	0	20
3						87
4	100	100		0	0	100
5			0			100
6	100	100			60	
7						
8	100	100		3	100	
9						
10	100	100	2		100	
12				30		
20			33	100		
30			93			
50			100			

¹Percent positive

²Based on 30 samples at each concentration

³Parent drug

**MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS ¹
SULFONAMIDES**

CHARM II SULFA DRUG TEST (COMPETITIVE ASSAY)¹

SCREENING TEST	SULFADIMETHOXINE	SULFAMETHAZINE	SULFATHIAZOLE	SULFADIAZINE
CHARM II SULFA DRUG TEST (COMPETITIVE ASSAY)	4.0	9.4	7.3	4.9

¹ Parts per billion (ppb), which can be detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following table. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.

CHARM II SULFA DRUG TEST (COMPETITIVE ASSAY) ^{1,2}

DRUG	SULFADIMETHOXINE	SULFAMETHAZINE	SULFATHIAZOLE	SULFADIAZINE
TOLERANCE/SAFE LEVEL (ppb)	10	10	10	10
DRUG CONCENTRATION (PPB)				
2	97	7	0	40
4	100	80	57	100
6	100	97	100	100
8	100	100	100	100
10	100	100	100	97

¹ Percent positive

² Based on 30 samples at each concentration

**MILK DRUG RESIDUE SCREENING TEST DETECTION CONCENTRATIONS ¹
TETRACYCLINES**

**CHARM II TETRACYCLINE DRUG TEST (COMPETITIVE
90/95 PERCENT DETECTION LEVELS (ppb))**

DRUG	90/95% DETECTION LEVELS (ppb)
Chlortetracycline	257
Oxytetracycline	119
Tetracycline	67

¹ Parts per billion (ppb), which can be, detected 90% of the time with 95% confidence. Additional drug level response data are provided for each test in the following tables and should be considered when selecting drug residue monitoring tests. The 90/95% concentrations (ppb) were determined by fitting a statistical model to the dose response data designed to estimate this value. The lower, one-sided 95% confidence limit was used. This data was either collected at an independent laboratory or the test samples were prepared at an independent laboratory.

CHARM II TETRACYCLINE DRUG TEST (COMPETITIVE)

DRUG	Chlortetracycline	Oxytetracycline	Tetracycline
TOLERANCE/SAFE LEVEL (ppb)	300 ppb (Chlortetracycline + Tetracycline + Oxytetracycline)		
DRUG CONCENTRATION (ppb)			
20			0
30			7
40			37
60		13	93
70		37	
90	17		
100		87	
120	20		
150		100	
160	77		
230	93		
300	97	100	100